

Amendment to the Claims

This listing of claims will replace all prior version, and listings, of claims in the application.

Listing of Claims

1. (Withdrawn) A method for cleaving single-stranded nucleic acid sequences at a desired location, the method comprising the steps of:

- (i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and
- (ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a

restriction endonuclease that is active at the chosen temperature.

2. (Withdrawn) A method for cleaving single-stranded nucleic acid sequences at a desired location, the method comprising the steps of:

- (i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a Type II-S restriction endonuclease recognition site, whose cleavage site is located at a known distance from the recognition site; and
- (ii) cleaving the nucleic acid solely at the Type II-S cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a

restriction endonuclease that is active at the chosen temperature.

3. (Withdrawn) In a method for displaying a member of a diverse family of peptides, polypeptides or proteins on the surface of a genetic package and collectively displaying at least a part of the diversity of the family, the improvement being characterized in that the displayed at least a part of peptide, polypeptide or protein is encoded at least in part by a nucleic acid that has been cleaved at a desired location by a method comprising the steps of:

- (i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and
- (ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large

enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

4. (Withdrawn) In a method for displaying a member of a diverse family of peptides, polypeptides or proteins on the surface of a genetic package and collectively displaying at least a part of the diversity of the family, the improvement being characterized in that the displayed peptide, polypeptide or protein is encoded by a DNA sequence comprising a nucleic acid that has been cleaved at a desired location by

- (i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a Type II-S restriction endonuclease recognition site, whose cleavage site is located at a known distance from the recognition site; and
- (ii) cleaving the nucleic acid solely at the Type II-S cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

5. (Withdrawn) A method for displaying a member of a diverse family of peptides, polypeptides or proteins on the surface of a genetic package and collectively displaying at least a part of the diversity of the family, the method comprising the steps of:

- (i) preparing a collection of nucleic acids that code at least in part for members of the diverse family;
- (ii) rendering the nucleic acids single-stranded;
- (iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:
 - (a) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location;
 - and

(b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature; and

(iv) displaying a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids on the surface of the genetic package and collectively displaying at least a portion of the diversity of the family.

6. (Withdrawn) A method for displaying a member of a diverse family of peptides, polypeptides or proteins on the surface of a genetic package and collectively displaying at least a portion of the diversity of the family, the method comprising the steps of:

- (i) preparing a collection of nucleic acids that code, at least in part, for members of the diverse family;
- (ii) rendering the nucleic acids single-stranded;
- (iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a Type II-S restriction endonuclease recognition site, whose cleavage site is located at a known distance from the recognition site; and

(b) cleaving the nucleic acid solely at the Type II-S cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the chosen temperature; and

(iv) displaying a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids on the surface of the genetic package and collectively displaying at least a portion of the diversity of the family.

7. (Currently Amended) A library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and collectively display at least a portion of the diversity of the family, ~~the library being produced using the methods of claims 3, 4, 5 or 6.~~ the displayed peptides, polypeptides or proteins being encoded at least in part by a nucleic acid that has been cleaved at a desired location by a method comprising the steps of:

(i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location;
and

(ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that

cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

8. (Original) A library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and that collectively display at least a portion of the family, the displayed peptides, polypeptides or proteins being encoded by DNA sequences comprising at least in part sequences produced by cleaving single-stranded nucleic acid sequences at a desired location by a method comprising the steps of:

(i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in

substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

9. (Original) A library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and that collectively display at least a portion of the diversity of the family of the displayed peptides, polypeptides or proteins being encoded by DNA sequences comprising at least in part sequences produced by cleaving single-stranded nucleic acid sequences at a desired location by a method comprising the steps of:

- (i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a Type II-S restriction endonuclease recognition site, whose cleavage site is located at a known distance from the recognition site where the cleavage of the nucleic acid is desired; and
- (ii) cleaving the nucleic acid solely at the Type II-S cleavage site formed by the

complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

10. (Currently amended) The ~~methods~~ library according to any one of claims ~~1 to 9~~ 7-9 and 42-44, wherein the nucleic acids encode at least a portion of an immunoglobulin.

11. (Currently amended) The ~~methods~~ library according to claim 10, wherein the immunoglobulin comprises a Fab or single chain Fv.

12. (Currently amended) The ~~methods~~ library according to claim 10 or 11, wherein the immunoglobulin comprises at least portion of a heavy chain.

13. (Currently amended) The ~~methods~~ library according to claim 12, wherein at least a portion of the heavy chain is human.

14. (Currently amended) The ~~methods~~ library according to claim 10 or 11, wherein the immunoglobulin comprises at least a portion of FR1.

15. (Currently amended) The ~~methods~~ library according to claim 14, wherein at least a portion of the FR1 is human.

16. (Currently amended) The ~~methods~~ library according to claim 10 or 11, wherein the immunoglobulin comprises at least a portion of a light chain.

17. (Currently amended) The ~~methods~~ library according to claim 16, wherein at least a portion of the light chain is human.

18. (Currently amended) The ~~methods~~ library according to any one of claims ~~1 to 9~~ 7-9 and 42-44, wherein the nucleic acid sequences are at least in part derived from patients suffering from at least one autoimmune disease and/or cancer.

19. (Currently amended) The ~~methods~~ library according to claim 18, wherein the autoimmune disease is selected from the group comprising lupus, erythematosus, systemic sclerosis, rheumatoid arthritis, antiphospholipid syndrome or vasculitis.

20. (Currently amended) The ~~methods~~ library according to claim 18, wherein the nucleic acids are at least in part isolated from the group comprising peripheral blood cells, bone marrow cells spleen cells or lymph node cells.

21. (Withdrawn) The methods according to claim 5 or 6 further comprising an nucleic acid amplification step between steps (i) and (ii), between steps (ii) and (iii) or between steps (iii) and (iv).

22. (Withdrawn) The methods according to claim 21, wherein the amplification step uses generACE™.

23. (Currently amended) The ~~methods~~ library according to any one of claims ~~1 to 9~~ 7-9 and 42-44, wherein the temperature is between 45°C and 75°C.

24. (Currently amended) The ~~methods~~ library according to claim 23, wherein the temperature is between 50°C and 60°C.

25. (Currently amended) The ~~methods~~ library according to claim 24, wherein the temperature is between 55°C and 60°C.

26. (Currently amended) The ~~methods~~ library according to claim ~~1, 3, 5 or~~ 8, wherein the length of the single-stranded oligonucleotide is between 17 and 30 bases.

27. (Currently amended) The ~~methods~~ library according to claim 26, wherein the length of the single-stranded oligonucleotide is between 18 and 24 bases.

28. (Currently amended) The ~~methods~~ library according to claim ~~1, 3, 5 or~~ 8, wherein the restriction endonuclease is selected from the group comprising *MaeIII*, *Tsp45I*, *HphI*, *BsaJI*, *AluI*, *BlpI*, *DdeI*, *BglII*, *MslI*, *BsiEI*, *EaeI*, *EagI*, *HaeIII*, *Bst4CI*, *HpyCH4III*, *HinfI*, *MlyI*, *PleI*, *MnlI*, *HpyCH4V*, *BsmAI*, *BpmI*, *XmnI*, or *SacI*.

29. (Currently amended) The ~~methods~~ library according to claim 28, wherein the restriction endonuclease is selected from the group comprising *Bst4CI*, *TaaI*, *HpyCH4III*, *BlpI*, *HpyCH4V* or *MslI*.

30. (Currently amended) The ~~methods~~ library according to claim ~~2, 4, 6 or~~ 9, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 14 and 22 bases.

31. (Currently amended) The ~~methods~~ library according to claim 30, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 14 and 17 bases.

32. (Currently amended) The ~~methods~~ library according to claim 31, wherein the length of the single-stranded region of the oligonucleotide is between 18 and 20 bases.

33. (Currently amended) The ~~methods~~ library according to claim ~~2, 4, 6 or~~ 9, wherein the length of the double-stranded region of the partially double-stranded oligonucleotide is between 10 and 14 base pairs formed by a stem and its palindrome.

34. (Currently amended) The ~~methods~~ library according to claim 33 wherein, the partially double-stranded oligonucleotide comprises a loop of 3 to 8 bases between the stem and the palindrome.

35. (Currently amended) The ~~methods~~ library according to claim ~~2, 4, 6 or~~ 9, wherein the Type II-S restriction endonuclease is selected from the group comprising AarICAC, AceIII, Bbr7I, BbvI, BbvII, Bce83I, BceAI, BceFI, BciVI, BfiI, BinI, BscAI, BseRI, BsmFI, BspMI, EciI, Eco57I, FauI, FokI, GsuI, HgaI, HphI, MboII, MlyI, MmeI, MnlI, PleI, RleAI, SfaNI, SspD5I, Sth132I, StsI, TaqII, Tth111II, or UbaPI.

36. (Currently amended) The ~~methods~~ library according to claim 35, wherein the Type II-S restriction endonuclease is FokI.

37. (Withdrawn) A method for preparing single-stranded nucleic acids for cloning into an vector, the method comprising the steps of:

(i) contacting a single-stranded nucleic acid sequence that has been cleaved with a restriction endonuclease with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region that remains after cleavage, the double-stranded region of the oligonucleotide including any sequences necessary to return the sequences that remain after cleavage into proper and original reading frame for expression and containing a restriction endonuclease recognition site 5' of those sequences; and
(ii) cleaving the partially double-stranded oligonucleotide sequence solely at the restriction endonuclease recognition site contained within the double-stranded region of the partially double-stranded oligonucleotide.

38. (Withdrawn) The method according to claim 37, wherein the length of the single-stranded portion of the partially double-stranded oligonucleotide is between 2 and 15 bases.

39. (Withdrawn) The method according to claim 38, wherein the length of the single-stranded portion of the partially double-stranded oligonucleotide is between 7 and 10 bases.

40. (Withdrawn) The method according to claim 37, wherein the length of the double-stranded portion of the partially double-stranded oligonucleotide is between 12 and 100 base pairs.

41. (Withdrawn) The method according to claim 40, wherein the length of the double-stranded portion of the partially double-stranded oligonucleotide is between 20 and 100 base pairs.

42. (New) A library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and collectively display at least a portion of the diversity of the family the displayed peptides, polypeptides or proteins being encoded at least in part by a nucleic acid that has been cleaved at a desired location by a method comprising the steps of:

(i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a Type II-S restriction endonuclease recognition site,

whose cleavage site is located at a known distance from the recognition site; and
(ii) cleaving the nucleic acid solely at the Type II-S cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

43. (New) A library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and collectively display at least a portion of the diversity of the family the displayed peptides, polypeptides or proteins being encoded at least in part by a nucleic acid that has been cleaved at a desired location by a method comprising the steps of:

- (i) preparing a collection of nucleic acids that code at least in part for members of the diverse family;
- (ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

- (a) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and
- (b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature; and

- (iv) displaying a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids

on the surface of the genetic package and collectively displaying at least a portion of the diversity of the family.

44. (New) A library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and collectively display at least a portion of the diversity of the family the displayed peptides, polypeptides or proteins being encoded at least in part by a nucleic acid that has been cleaved at a desired location by a method comprising the steps of:

(i) preparing a collection of nucleic acids that code, at least in part, for members of the diverse family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a Type II-S restriction endonuclease recognition site, whose cleavage site is located at a known distance from the recognition site; and

(b) cleaving the nucleic acid solely at the Type II-S cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the chosen temperature; and

(iv) displaying a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids on the surface of the genetic package and collectively displaying at least a portion of the diversity of the family.